Leptin expression in proliferative, secretory and hyperplastic endometrial tissues

Proliferatif, sekretuar ve hiperplazik endometrial dokuda leptin ekspresyonu

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Abstract

Objective: The goal of this study was to detect endometrial leptin expression in proliferative and secretory phases and then to compare the results with that of hyperplastic endometrium.

Material and Methods: Seventeen proliferative, 23 secretory phase and 18 hyperplastic endometrial tissues diagnosed in our hospital between 2002 and 2007 were included in the study. These samples were stained with leptin antibody using an immunohistochemical method. Endometrial glandular and surface epithelium and stroma were evaluated for staining distribution and intensity.

Conclusion: Staining intensity seen in early proliferative phase samples (2.33±0.51) increased significantly throughout the middle (2.40±0.54) and late phases (2.83±0.40) ($p<0.05$). Early secretory phase samples had the least staining intensity (1.42±0.53), while it increased significantly in later periods (2.38±0.51) ($p<0.05$). There was no difference in staining intensity among proliferative, secretory and hyperplastic tissues ($p>0.05$).

Conclusion: Although endometrial leptin expression was observed in a differential manner throughout the whole menstrual period, no difference was seen in endometrial hyperplasia. We consider that leptin does not play a role in hyperplastic transformation of the endometrium. (J Turkish-German Gynecol Assoc 2011; 12: 157-61)

Key words: Endometrium, hyperplasia, leptin, menstrual period, phase

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Özet

Amaç: Bu çalışmanın amacı proliferasyon fazı ve sekresyon fazı endometrium dokularında leptin ekspresyonu varlığını araştırmak ve çıkan sonuçları hiperplastik endometrium dokularından elde edilen sonuçlarla karşılaştırmaktır.

Gereç ve Yöntemler: Hastanemizde 2002-2007 yılları arasında tanı almış 17 adet proliferasyon fazında endometrium, 23 adet sekresyon fazında endometrium ve 18 adet endometrial hiperplazi dokusu çalışmaya dahil edildi. Bu örnekler leptin antikorları ile immünohistokimyasal metod kullanılarak boyandı. Endometrial bez yapıları, yüzey epiteli ve stroması boyanma derecesi ve dağılımı açısından değerlendirildi.

Bulgular: Boyanma derecesi erken proliferatif faz örneklerinde (2.33±0.51) belirgin olarak az olduğu, orta (2.40±0.54) ve geç proliferasyon fazlarına (2.83±0.40) doğru belirgin olarak arttığı gözlendi. Boyanma derecesi erken sekresyon fazı örneklerinde (1.42±0.53) en düşüktü fakat daha geç sekretuar fazlarda (2.38±0.51) belirgin olarak arttığı gözlendi ($p<0.05$). Proliferatif, sekretuar ve hiperplastik dokular arasında leptin ekspresyonu açısından istatiksel anlamda bir fark tespit edilmedi ($p<0.05$).

Sonuçlar: Adet döngüsü boyunca endometrial leptin ekspresyonu açısından anlamlı farklılıklar gözlenmesine ragmen endometrial hiperplazide anlamlı farklılık gözlenmedi. Endometriumun hiperplastik transformasyon sürecinde leptinin etkisinin olmadığını düşündürmüştür. (J Turkish-German Gynecol Assoc 2011; 12: 157-61)

Anahtar kelimeler: Endometrial hiperplazi, leptin, adet döngüsü

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Introduction

Following the discovery of the ob/ob mouse model in 1950, mutational changes were reported to cause serious obesity, hyperphagia, diabetes and reduced energy consumption at relatively early ages. The genetic defect leading to the ob/ob mouse was published in 1994 (1) and leptin, which is stimulated with 16-k Da ob gene and is mainly synthesized by adipose tissue, was introduced as an ob gene product in 1995 (2, 3). Leptin regulates body weight and energy balance by interacting with its receptors found in various tissues. Six different isoforms of leptin receptor were identified (4). In recent studies, leptin was reported to play major roles in many reproductive events such as menstruation, ovarian follicle maturation, embryo development and continuation of gestation (5-7). In addition, expression of OB-R_L mRNA was detected in human endometrium (8), which is a target organ for leptin (9). Insulin-like growth factor-1 (IGF-1) and leptin and were found to be related with proliferation and mitogenic activity in rat mammarian gland cell cultures and to play a role in the pathogenesis of breast cancer (10). Patients with endometrial hyperplasia and endometrial cancer had higher serum leptin levels when compared with controls with adjusted body mass index (BMI) values, and leptin was reported

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to have a significant effect on endometrial proliferation (11). Leptin's effect on proangiogenic molecules (VEGF, IL-1beta, LIF) and their receptors (VEGFR2, IL-1R, LIFR) was much more prominent in endometrial cancer cells compared with benign endometrial tumors, and leptin and/or a pathogenic pathway stimulated by leptin were postulated to be responsible for this malignant transformation (12). It has been considered that leptin might affect endometrial proliferation and its receptors might be regulated by some proliferative factors. Our goal in this study was to detect leptin staining in proliferative phase endometrium (PPE), secretory phase endometrium (SPE) and in tissues with endometrial hyperplasia (EH) and then to compare staining intensities by using an immunohistochemical method.

Materials and Methods

Seventeen PPE, 23 SPE and 18 EH tissues, diagnosed and kept in the Department of Pathology, Faculty of Medicine, Celal Bayar University between 2002-2007 were included in the study. Seven PPE, 6 SPE and 4 EH samples were obtained by probe curettage while 10 PPE, 17 SPE and 14 f EH samples were hysterectomy specimens. Demographic data such as age and menstrual irregularity were recorded from the patients' files. Probe curettage and hysterectomy indications were irregular bleeding and uterine fibroids. None of the patients had any additional disorders or received any medications prior to 1 month of surgical procedures. In the first step of the study, leptin expression was evaluated after PPE and SPE were individually divided into early, middle and late periods. In the second step of the study, leptin expression was evaluated in two different pathological states, simple and complex hyperplasia. Slides stained with hematoxyline and eosin were reevaluated by the pathologist who was not informed of the origin of the specimens, menstrual cycle days were determined and histopathological diagnosis was confirmed.

For immunohistochemical analysis, sections obtained from formalin-fixed and paraffin-embedded blocks were deparaffinized with xylene and rehydrated with graded ethanol. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 min. Slides were washed with distilled water for 5 min, placed in 0.01 M citrate buffer (pH 6.0) and boiled in the pressure cooker during 3 minutes. Background blocking was performed with 1:20 normal goat serum (Dako; Glostrup, Denmark) in 0.05 M Tris-HCl buffer, 0.5 M NaCl, pH 7.6 (TBS) before incubation with specific antiserum. The tissue sections were incubated at room temperature for 20 min with a rabbit polyclonal antibody specific for human leptin (Ob (A-20) sc-843; (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:75 in primary antibody diluent (K004, LOT G537 Pleasanton, CA). After 20 min incubation with the linker (biotin), streptavidin-peroxidase was added for an additional 20 min and the substrate-chromogen solution (DAB) was used for 5 min to stain the slides. Subsequent to each incubation step, the tissues were washed three times with PBS 50 mm Tris-HCl buffer. Counterstaining was carried out with Mayer's hematoxylin, and the slides were mounted with Entellan (Merck and Co., Berlin). A section of placental sample was used as positive control for each staining procedure. All samples were evaluated by one pathologist under standard light microscope and described as follows: Endometrial glands and surface epithelium: 0: negative, 1: minimal staining, 2: mild staining, 3: strong staining. Stroma: 0: negative, 1: mild staining, 2: strong staining.

Kruskal-Wallis and Chi-square tests were used to compare staining intensities and $p<0.05$ was accepted as statistically significant.

Results

The ages of the patients were between 30 and 74 years. Clear cytoplasmic staining of leptin is seen in the glandular and surface epithelium of the endometrium and cytoplasmic and/or nuclear staining in endometrial stromal cells. The staining intensity was significantly different among early (2.33±0.51) (Fig. 1A), middle (2.40±0.54) (Fig. 1B) and late (2.83±0.4) (Fig. 1C) PPE samples ($p<0.05$) (Table 1). Leptin expression began to increase in early PPE and reached high intensity until ovulation. In early SPE, a nadir value was observed (1.42±0.53) (Fig. 2A), and then increased with time (2.00±1.00) (Fig. 2B) and reached its maximum level (2.38±0.51) (Fig. 2C) in late SPE samples ($p<0.05$) (Table 2).

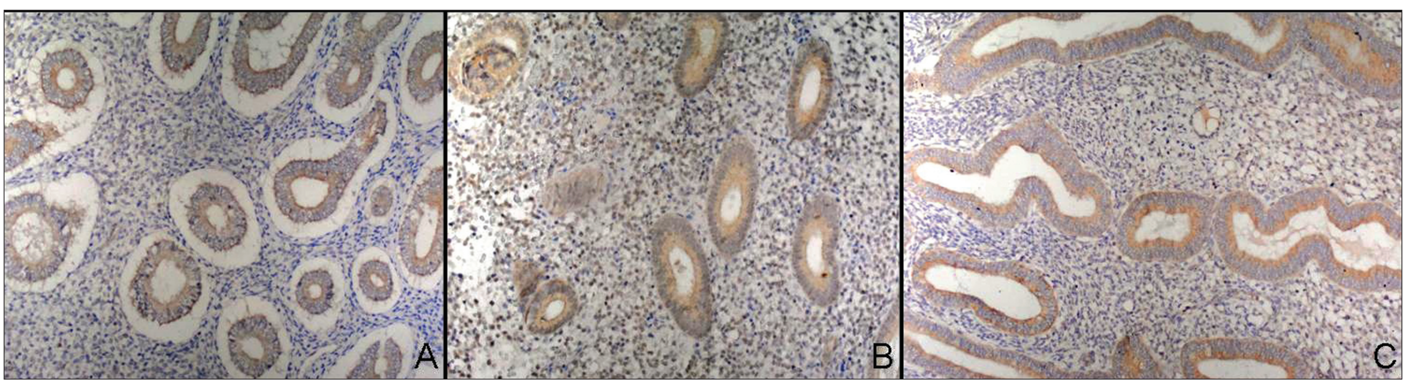

Figure 1. A: Leptin staining in endometrial gland epithelium and stromal cells in early proliferative phase (x200). B: Leptin staining in endometrial gland epithelium and stromal cells in middle proliferative phase (x200). C: Leptin staining in endometrial gland epithelium and stromal cells in late proliferative phase (x200). The strongest staining intensity is seen in this phase

Staining intensity was the same in simple and complex hyperplasia groups (2.5±0.59) (Figure 3). As the numbeof cases in complex EH was fewer and there was no difference between simple and complex groups, we joined these two groups under EH and then compared with normal PPE and SPE samples. There was no significant difference between these 3 groups (p>0.05) (Table 3).

Discussion

Obesity is accepted to be related with development, morbidity and mortality of colon, breast, endometrium, renal, pancreas and esophagus cancer (13). Increased waist circumference, commonly seen in obese patients, is a well-known risk factor for these disorders. While endometrium cancer is the fourth most common cancer in women, it is in the first place in the genital system. Endometrial hyperplasia is an important clinical entity as a precancerous lesion, with a serious potential to transform into cancer. If development of endometrial hyperplasia is prevented or cured in relatively early stages (for example at the simple hyperplasia level), endometrial cancer prevalence may be reduced.

Previous studies searching for expression of human endometrial leptin and its receptors revealed the presence of OB-R receptor mRNA while no sign was found for leptin expression (8, 9). Using Western blot analysis, the researchers suggested that the uterus was a target organ for leptin produced in adipose tissue but it was not expressed in the endometrium. However, in another study, Gonzalez et al. showed human endometrial leptin mRNA by RT-PCR and its protein by immunohistochemistry methods (5). In our present study, we revealed different levels of leptin expression in different phases of the human endometrium by using immunohistochemistry staining. Although we did not point out the leptin source, we could prove

Table 1. Leptin expression in PPE. The difference is statistically significant among the samples (p<0.05). (Numbers refer to mean±SD)

	# Patients	Staining Intensity
Early PPE	6	2.33±0.51
Mid PPE	6	2.40±0.54
Late PPE	5	2.83±0.40
Total	17	2.52±0.10

Table 2. Leptin expression in SPE. The difference is statistically significant among the samples (p<0.05). (Numbers refer to mean±SD)

	# Patients	Staining Intensity
Early SPE	7	1.42±0.53
Mid SPE	3	2.00±1.00
Late SPE	13	2.38±0.51
Total	23	2.04±0.71

Table 3. Leptin expression in PPE, SPE, EH. There was no statistically significant difference among the 3 groups (p>0.05). (Numbers refer to mean±SD)

	# Patients	Staining Intensity
PPE	17	2.52±0.51
SPE	23	2.04±0.70
EH	18	2.33±0.59
Total	58	2.27±0.64

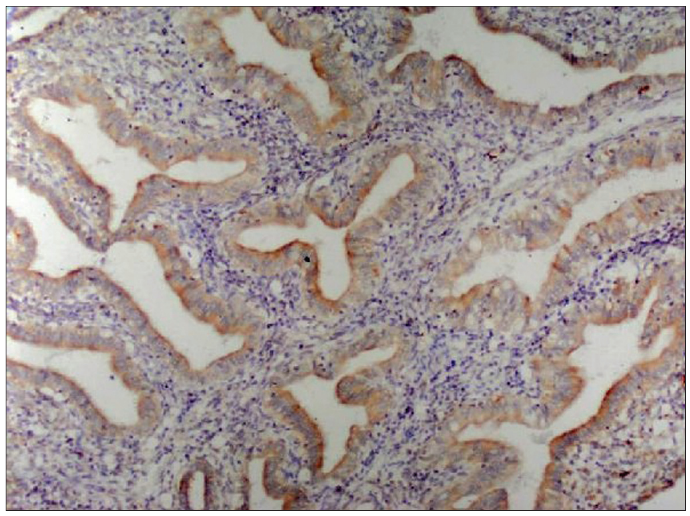

Figure 3. Leptin staining in endometrial gland epithelium and stroma in endometrial hylperplasia (x200)

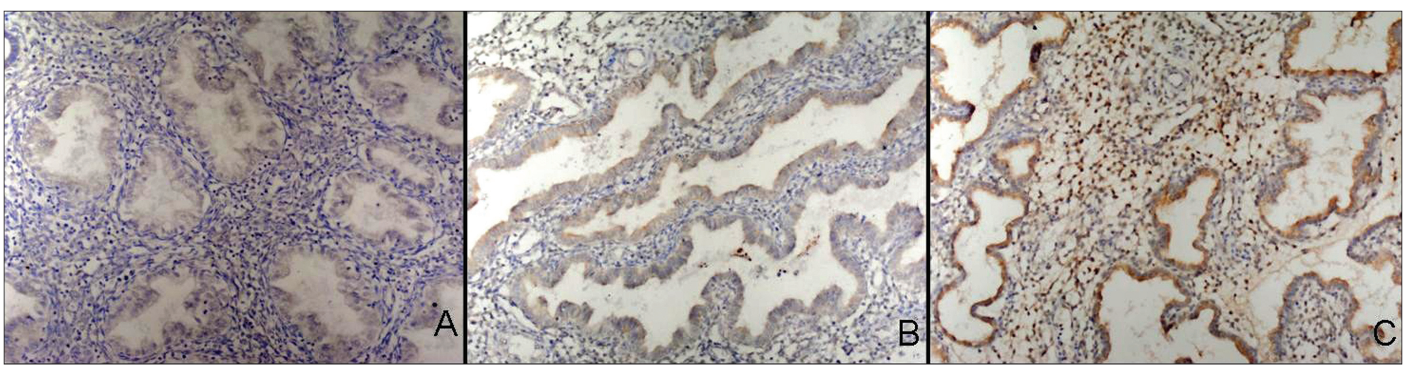

Figure 2. A: Leptin staining in endometrial gland epithelium and stromal cells in early secretory phase (x200). Mild staining in early period becomes stronger in time. B: Leptin staining in endometrial gland epithelium and stromal cells in middle secretory phase (x200). C: Leptin staining in endometrial gland epithelium and stromal cells in late secretory phase (x200)

its presence. It is accepted that leptin exists in human endometrium even though there is no consensus about whether it is produced within the endometrium or produced and secreted by adipose tissue, passes s via the vascular system and acts on the endometrium.

We found that leptin existed in an increasing level, beginning from the early to late proliferation phase and reaching a maximum level in the latter and then decreased just following ovulation in the early secretory phase. In a study investigating expression of leptin and its receptors throughout the complete menstrual period, very low OB-R_L mRNA expression was found in the beginning but it continuously increased during the remainder of the proliferative phase (9). This variable expression of the receptor is in accordance with leptin expression shown in our study. Serum leptin concentration was found to be well correlated with serum estradiol levels in a study searching for fluctation of leptin during a normal menstrual period (14). When the results of this study, which show that serum leptin increases at late proliferation and reaches a peak level at periovulatory phase are compared with our results, we can state that estradiol may act upon endometrial leptin expression and leptin may collaborate with estradiol to affect the endometrium. However, as in vitro studies have shown that estradiol does not have an acute effect on OB-R_L mRNA expression, it does not seem to take part in a direct action to increase endometrial leptin and OB-R expression. Elevation of leptin expression is in parallel to estradiol levels, however estradiol may act as a limiting factor for leptin action on endometrium by exerting no increase in receptor levels.

Following endometrial sloughing with menstrual bleeding, rapid repair commences and the endometrium prepares itself for a probable implantation. New endometrium covers 75% of the cavity on day 4, re-epithelization occurs on day 5-6 and stromal changes begin. The epithelization process accelerates with the increased vascular supply (angiogenesis). A positive correlation was determined between leptin and erythropoietin on endometrial bleeding and angiogenesis during a normal menstrual cycle (12). Proangiogenic molecules VEGF, IL-1-β, LIF and their receptors have been expressed more with leptin in both benign and malignant endometrial disorders (4). In another study, the effect of leptin on normal endometrial stromal cells was detected by the 8 Br-cAMP method (in vitro decidualization measurement system) and leptin was found to support viability and inhibit decidualization in a dose dependent manner (15). Leptin seems to play a role in differentiation of the endometrium from sloughing to implantation phases with such angiogenetic and anti-apoptotic effects reported in these studies. This result necessitates increased leptin concentration, beginning from early to late proliferative phases and a secondary increase from a nadir value at early to late secretory phase as shown in our study. The increased angiogenesis and anti-apoptotic effect in accordance with a rise in endometrial leptin expression from early to late proliferative phases elicit adequate endometrial proliferation. Embryo implantation is accepted as a phase mediated apoptosis (16, 17). Reduced leptin concentration in early SPE induces apoptosis and consequently implantation. During the late secretory phase, increased endometrial leptin expression is required for angiogenesis, which is the next step in implantation. All these relevant changes support the results of our study.

Patients with endometrial hyperplasia and cancer had significantly higher serum leptin concentration when divided into 3 groups according to their BMI levels and compared with women having normal endometrium (11). Angiogenetic and anti-apoptotic effects of leptin may be considered to participate in the pathophysiology of endometrial hyperplasia in addition to a physiologic proliferative effect on endometrium. However, when we compared staining intensities in PPE, SPE and EH groups, we could not demonstrate a significant difference in leptin expression. Although leptin may act upon cellular proliferation, it may not play a role in hyperplastic transition. A strong positive relation was reported between leptin and endometrial cancer in a case control study by Petridou et al. (18). In addition, short and long receptor isoforms of leptin were detected in endometrial cancer cells (19). In another study, proliferation and invasiveness of endometrial cancer cells were found to be enhanced by leptin (with JAK/STAT activation) (20). These studies may indicate that leptin may play a role in malignant transformation. Leptin receptor existence in cancerous tissues may give rise to leptin's role in the development of cancer even though it does not seem to participate in the hyperplasia stage. As we observed samples with established and manifest hyperplasia, we do not have information about leptin's effect during the transitional period from the normal to hyperplastic stage.

In conclusion, leptin may act upon endometrial changes seen in the proliferative and secretory phases, but as it is not expressed in different amounts in hyperplastic tissues, we consider that it does not participate in this pathological procedure. Different results may be expected in a long term, prospective study which follows obese women with histopathologically proven normal endometrium and high serum leptin levels and women having normal BMI and normal serum leptin. Such a study may t expose a probable effect of leptin on the endometrial neoplastic process with more objective criteria.

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Conflict of interest
No conflict of interest exists among the authors for any particular subject.

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